UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 14-FEB-2000

SUBJECT: **Prohexadione-Calcium.** Issues to be Presented to the HED Metabolism Assessment Review Committee Meeting on 2/22/00. Barcode D262930. Chemical 112600. Case 289440. Submission S543993.

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THROUGH: Melba Morrow, Branch Senior Scientist
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TO: HED Metabolism Assessment Review Committee Members[1]

K-I Chemical U.S.A. has submitted a petition for the establishment of permanent tolerances for residues of a new plant growth regulator, prohexadione-calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), in/on peanuts, pome fruits, and cattle meat byproducts (kidney). Concurrently, the petitioner is requesting Section 3 registration for end-use products containing prohexadione-calcium as the active ingredient; a 27.5% dry flowable (DF) formulation (Product Name = Apogee™, EPA File Symbol No. 63588-RR); and a 75% DF formulation (Product Name = Baseline®, EPA File Symbol No. 63588-O). Apogee™ is intended for use on apple and pear trees and Baseline™ is intended for use on peanuts. Prohexadione-calcium is effective in controlling vegetative growth in pome fruits and peanuts by inhibiting gibberellin biosynthesis. In peanuts, prohexadione-calcium is effective in canopy control, which facilitates row location for mechanical harvesting; in pome fruits, prohexadione-calcium improves fruit quality by allowing increased sunlight penetration

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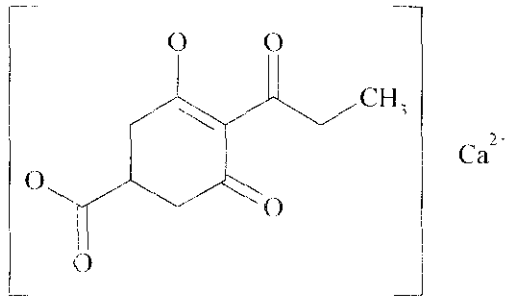
into the canopy. Section F of the petition proposes the establishment of tolerances for residues of prohexadione-calcium *per se* in/on the following commodities:

Peanut Nutmeat	1.0 ppm
Peanut Hay	0.6 ppm
Pome fruit	3.0 ppm
Cattle, Meat Byproduct (Kidney)	0.1 ppm

The structure of prohexadione-calcium is shown below:



1. RESIDUE CHEMISTRY DATA

Proposed Use.

The petitioner provided a specimen label for the 27.5% DF formulation [Product Name = Apogee™; EPA File Symbol No. 63588-RR] containing prohexadione-calcium as the active ingredient, and which is proposed for use on pome fruits. The 27.5% DF formulation is proposed for single, split, or multiple foliar applications to apple and pear trees at 0.206-0.825 lb ai/A/application with a maximum seasonal application rate of 1.70 lb ai/A/year. Retreatment intervals of 7 to 17 days are recommended; however, application of more than 0.825 lb ai/A within any 21-day interval is prohibited. A 45-day preharvest interval (PHI) is proposed. A restricted entry interval (REI) of 12 hours is proposed.

The petitioner provided a specimen label for the 75% DF formulation [Product Name = Baseline™; EPA File Symbol No. 63588-O] containing prohexadione-calcium as the active ingredient, and which is proposed for use on peanuts. The 75% DF formulation is proposed for a maximum of three broadcast foliar applications at 0.125 lb ai/A/application with a 2-4 week retreatment interval using ground equipment. A maximum seasonal rate of 0.375 lb ai/A and a 25-day PHI are proposed. Rotational crop restrictions were not included on the Baseline™ label. However, HED has concluded that a 30-day plantback restriction is appropriate for the purpose of this petition (see below).

Enforcement Method.

To measure residues of prohexadione-calcium in plants, the petitioner has developed residue analytical methods using GC and a mass selective detector (GC/MSD). These GC/MSD methods are designated Methods D9601 and D9608. Method D9601 was first developed to measure residues of prohexadione-calcium in/on peanut nutmeat and hay. Method D9608 is identical to Method D9601, and was later developed to include instructions for the analysis of residues in/on pome fruits and livestock commodities. The methods are proposed for tolerance enforcement, and were used as the data-collection methods in the analyses of samples obtained from the field, processing, and storage stability studies. Both methods were designed to measure residues of prohexadione-calcium as the prohexadione methyl ester. The reported level of quantitation (LOQ) for prohexadione-calcium is 0.05 ppm for all apple, pear, and peanut commodities. The method has been sent to the Agency Analytical Chemistry Laboratories (ACL) in Fort Meade for a Petition Method Validation (PMV) (Memo, G. Kramer 8/3/99; D257929).

The petitioner proposes Method D9608 as an animal enforcement method. The method can determine residues of prohexadione-calcium and despropionyl prohexadione in livestock commodities. Quantitation of prohexadione-calcium is by GC/MSD, and quantitation of the despropionyl metabolite is by HPLC/UV. The reported LOQ for prohexadione-calcium was 0.05 ppm for liver, kidney, fat, and muscle and 0.01 ppm for milk. The reported LOQ for despropionyl prohexadione was 0.05 ppm for liver and kidney.

The petitioner submitted data (MRID 44457802) concerning the recovery of residues of prohexadione and its despropionyl metabolite using FDA Multiresidue method protocols (PAM Vol. I). Residues of prohexadione and the despropionyl metabolite are not recovered using FDA Multiresidue method protocols.

Nature of the Residue.

Apple:
Total radioactive residues (TRRs) were 0.305 ppm in/on mature apples harvested 45 days following the last of two sequential applications of [^{14}C]prohexadione-calcium, labeled at the C-3 and C-5 positions of the cyclohexenone ring, at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate). Applications were made directly to apples as a run-off spray. Prohexadione-calcium was rapidly metabolized to prohexadione (free acid), which was identified at 1.83% TRR (0.0056 ppm) (Table 1). Metabolites BX 112-I5 and BX 112-M10 were the major identified metabolites, accounting for 11.78% TRR (0.0359 ppm) and 9.21% TRR (0.0281 ppm), respectively. The following metabolites were also identified: despropionyl prohexadione (5.55% TRR, 0.0169 ppm),

27F2-B (7.68% TRR, 0.0234 ppm), 25F1-A (5.33% TRR, 0.0163 ppm), 27F2-A (2.60% TRR, 0.0079 ppm), tricarballylic acid (1.24% TRR, 0.0038 ppm), and citric acid (2.44% TRR, 0.0074 ppm). Organosoluble unknowns (20 components) accounted for 36.32% TRR and aqueous-soluble unknowns (6 components) accounted for 5.92% TRR. The petitioner demonstrated that the remainder of the characterized radioactivity was distributed between base hydrolysate unknowns <3000 MW (5 components) at 1.32% TRR, base hydrolysate unknowns >3000 MW at 1.29% TRR, and glucose osazones at 2.20% TRR. The chemical structures of metabolites identified in plant and animal metabolism studies are depicted in Figure 1 (Attachment I).

Table 1. Summary of radioactive residues characterized/identified in apples harvested 45 days following treatment with [^{14}C]prohexadione-calcium at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate).

Fraction	Apple - 45-DAT (TRR = 0.305 ppm)	
	% TRR	ppm
Identified [a]		
Prohexadione	1.83	0.0056
BX 112-I5	11.78	0.0359
BX 112-M10	9.21	0.0281
Despropionyl prohexadione	5.55	0.0169
27F2-B (and 45F2-A)	7.68	0.0234
25F1-A	5.33	0.0163
27F2-A (and 27F1-A)	2.60	0.0079
TCA	1.24	0.0038
Citric acid	2.44	0.0074
Total identified	**47.66**	**0.1453**
Characterized		
Unknown 6F1 (polar)	0.59	0.0018
Unknown 9F (polar)	4.40	0.0134
Unknown 13F (polar)	3.60	0.0110
Peak 5	1.86	0.0057
Unknowns 19F1, 19F2, 19F4	5.46	0.0166
Unknown 25F4 (4 components)	1.43	0.0044
Peak 12	2.28	0.0070
Unknown 33F	5.49	0.0167
Unknown 35F (5 components)	6.22	0.0190
Peak 17	1.88	0.0057
Peak 18	3.11	0.0095
Aqueous soluble unknowns (6)	5.92	0.0181
Base hydrolysate unknowns <3000 MW (5, each <0.50% TRR)	1.32	0.0040
Base hydrolysate unknowns >3000 MW	1.29	0.0039
Glucose osazones	2.20	0.0067
Total identified/characterized	**94.71**	**0.2888**
Nonextractable [b]	2.21	0.0067

[a] See Figure 1 for names and structures of identified metabolites.
[b] Calculated by difference.

Peanut:

TRRs were 4.15 ppm in peanut nutmeats, 2.50 ppm in hulls, and 36.5 ppm in hay collected 22 days following an over-the-top postemergence spray of [^{14}C]prohexadione-calcium labeled at the C-3 and C-5 positions of the cyclohexenone ring at ~1.0 lb ai/A (~2.7x the maximum proposed seasonal rate of 0.375 lb ai/A). Over 70% (nutmeats , 52% (hulls), and 61% (hay) of the TRR were characterized and identified (Table 2). Prohexadione-calcium was rapidly metabolized to prohexadione (free acid). Prohexadione was the major residue identified in nutmeats (38.3% TRR, 1.58 ppm), hulls (9.66% TRR, 0.24 ppm), and hay (35.08% TRR, 12.80 ppm). The dioxopropyl prohexadione metabolite was identified in hulls (7.48% TRR, 0.19 ppm), nutmeats (2.08% TRR, 0.09 ppm) and hay (5.79% TRR, 2.12 ppm). The following additional metabolites were identified: despropionyl prohexadione in hulls (2.54% TRR, 0.064 ppm) and hay (1.69% TRR, 0.615 ppm); and TCA in nutmeats (3.06% TRR, 0.13 ppm), hulls (11.92% TRR, 0.40 ppm), and hay (12.66% TRR, 4.62 ppm).

Two unknowns, HA5 and HA6, characterized as oxidation products similar to dioxopropyl prohexadione accounted, respectively, for 5.63% and 6.01% TRR in nutmeats, 2.45% and 1.84% TRR in hulls, and 1.27% and 1.15% TRR in hay. In addition, residues characterized as lipids accounted for 3.01% TRR in nutmeats; residues characterized as lignin accounted for 2.61% and 3.70% TRR, respectively, in hulls and hay. The nonextractable residues in nutmeats, hulls, and hay were characterized as base-labile conjugates of prohexadione and its metabolites, sugars (osazones), carbohydrates, proteins, and polar metabolites <3000 Molecular Weight.

Table 2. Summary of radioactive residues characterized/identified in peanut commodities harvested 22 days following treatment with [^{14}C]prohexadione-calcium at a total application rate of ~1 lb ai/A (~2.7x the maximum proposed seasonal rate).

Fraction	Nutmeats (TRR = 4.15 ppm)		Hulls (TRR = 2.50 ppm)		Hay (TRR = 36.5 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Prohexadione	38.3	1.58	9.66	0.24	35.08	12.80
Dioxopropyl prohexadione [b]	2.08	0.09	7.48	0.19	5.79	2.12
TCA	3.06	0.13	15.92	0.40	12.66	4.62
Total Identified	**43.44**	**1.80**	**33.06**	**0.83**	**53.53**	**19.54**
Characterized [c]						
HA5	5.63	0.23	2.45	0.06	1.27	0.46
HA6	6.01	0.25	1.84	0.05	1.15	0.42
NMC2	1.37	0.057	--	--	--	--
NMC3	1.00	0.042	--	--	--	--
NMC4	3.88	0.161	--	--	--	--
NA1	2.10	0.087	--	--	--	--
HLMC4	--	--	0.55	0.01	--	--
HLA1	--	--	2.18	0.06	--	--
HLA2	--	--	4.59	0.12	--	--
HLA6	--	--	4.70	0.12	--	--
HH1	--	--	--	--	0.01	<0.01
Lipids	3.01	0.13	0.01	<0.001	0.02	0.01
Lignin	--	--	2.61	0.06	3.70	1.35
Polar (>3000 MW)	3.93	0.16	0.20	0.01	1.51	0.550
Total identified/characterized	**70.37**	**2.92**	**52.19**	**1.32**	**61.19**	**22.33**
Nonextractable	1.10	0.05	18.3	0.46	9.69	3.53

[a] See Figure 1 for chemical names and structures of identified metabolites. Although structures were not proposed for HA5 and HA6, the petitioner concluded that these are oxidation products similar to dioxopropyl prohexadione.

[b] Despropionyl prohexadione was only identified in the base hydrolysate of nonextractable residues following buffer extraction at 2.54% TRR (hulls) and 1.69% TRR (hay).

[c] The petitioner conducted additional procedures on nonextractable residues following solvent extraction which characterized residues as base-labile conjugates, sugars (osazones), carbohydrates, proteins, and polar metabolites <3000 MW.

Plant metabolism conclusions:

The qualitative nature of the residue of prohexadione-calcium in plants is adequately understood for the purpose of this petition. The metabolism of prohexadione-calcium in apples and peanuts is similar (Figures 2 and 3). Prohexadione-calcium is rapidly metabolized to prohexadione and parent-like oxidative intermediates and ultimately to tricarballylic acid (TCA), citric acid, and other natural products from the plant carbon pool.

Rotational Crops:

[^{14}C]Prohexadione-calcium was applied to sandy loam soil at 0.343 lb ai/A (~1x the maximum proposed seasonal application rate for peanuts). Representatives of small grains (wheat), leafy vegetables (lettuce), and root crops (turnips) were planted 31- and 122-days after treatments (DAT). TRRs were below <0.01 ppm in/on all rotational crop commodities except in 31-DAT wheat grain (0.0141 ppm), 31-DAT wheat straw (0.0237 ppm), and 122-DAT wheat straw (0.0188 ppm).

Rotational crop commodities with total radioactivity greater than 0.01 ppm were subjected to extraction and hydrolysis procedures in order to characterize/identify residues. No residues of prohexadione-calcium or related metabolites were identified. Enzyme hydrolyses demonstrated that the majority of the radioactivity was associated with carbohydrates (120% TRR, 0.017 ppm) in wheat grain, and either carbohydrate associated (27.9% TRR, 0.007 ppm) or nonextractable (37.2% TRR, 0.009 ppm) residues in wheat straw. All extracts other than those characterized by enzyme hydrolysis, and nonextractable residues were ≤0.01 ppm and did not require additional characterization/identification.

Based on the results of this study, HED concludes that limited rotational field studies are not required and a 30-day plantback restriction is appropriate for the purpose of this petition.

Ruminants:

Following oral administration of [^{14}C]prohexadione-calcium to lactating goats for four consecutive days at 105 ppm, the TRR were <0.0604-0.0884 ppm in milk, 3.09-3.16 ppm in kidney, 0.427-0.432 ppm in liver, 0.061-0.069 ppm in muscle, and 0.048-0.054 ppm in fat. The feeding level of 105 ppm is equivalent to ~52x the anticipated maximum dietary burden of 2.0 ppm for dairy cattle and ~31x the anticipated maximum dietary burden of 3.4 ppm for beef cattle.

Prohexadione was the major residue identified in milk (19.0% TRR, 0.016 ppm), kidney (40.9% TRR, 1.267 ppm), liver (30.6% TRR, 0.131 ppm), muscle (73.5% TRR, 0.051 ppm), and fat (89.3% TRR, 0.043 ppm) (Table 3). Metabolites related to, or a precursor of, despropionyl prohexadione were identified in kidney (31.7% TRR, 0.980 ppm) and liver (10.5% TRR, 0.045 ppm). Polar and low molecular weight metabolites were characterized as carboxylic acids in liver (20.2% TRR, 0.086 ppm). Milk contained radioactivity incorporated into sugars (22.6% TRR, 0.019 ppm) and lipids (15.2% TRR, 0.012 ppm), suggesting metabolism of prohexadione to the carbon pool.

Table 3. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat orally dosed with [^{14}C]prohexadione-calcium at 105 ppm for 4 consecutive days.

Fraction	Milk, Day 4 (TRR = 0.082 ppm)		Kidney (TRR = 3.09 ppm)		Liver (TRR = 0.427 ppm)		Muscle (TRR = 0.069 ppm)		Fat (TRR = 0.048 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]										
Prohexadione	19.0	0.016	40.9	1.267	30.6	0.131	73.5	0.051	89.3	0.043
Despropionyl prohexadione and precursor metabolites	--	--	31.7	0.980	10.5	0.045	--	--	--	--
Total identified	**19.0**	**0.016**	**72.6**	**2.247**	**41.1**	**0.176**	**73.5**	**0.051**	**89.3**	**0.043**
Characterized										
Low molecular weight carboxylic acids	--	--	--	--	20.2	0.086	--	--	--	--
Sugars	22.6	0.019	--	--	--	--	--	--	--	--
Lipids	15.2	0.012	--	--	--	--	--	--	--	--
Unknown peaks	14.2	<0.011	18.8	0.581	17.8	0.077	--	--	--	--
Aqueous	--	--	--	--	--	--	10.4	0.007	9.1	0.004
Petroleum ether extract	--	--	0.1	0.004	0.8	0.003	0.3	<0.001	4.5	0.002
>3000 MW	--	--	2.7	0.082	3.0	0.013	--	--	--	--
Aqueous (<3000 MW)	--	--	2.8	0.088	6.2	0.027	--	--	--	--
Methylene chloride (pronase hydrolysate)	--	--	1.6	0.049	2.2	0.009	--	--	--	--
Total identified and characterized	**71.0**	**0.058**	**98.6**	**3.051**	**91.3**	**0.391**	**84.2**	**0.059**	**102.9**	**0.049**
Nonextractable	17.4	0.014	1.8	0.055	2.2	0.009	17.2	0.012	11.1	0.005

[a] See Figure 1 for chemical names and structures of identified metabolites.

Poultry:

Following oral administration of [^{14}C]prohexadione-calcium to laying hens for five consecutive days at 8.43 ppm (~34x the maximum theoretical dietary burden for poultry), the TRR in eggs and tissues were <0.01 ppm each. In the same study, following oral administration of [^{14}C]prohexadione-calcium at 33.4 ppm (~134x), the TRRs were <0.008-0.019 ppm in egg yolks, <0.005-0.015 ppm in egg whites, 0.021 ppm in gizzard, 0.475-0.471 ppm in kidneys, 0.0289-0.030 ppm in liver, <0.007 ppm in breast muscle, 0.010-0.011 ppm in thigh muscle, <0.01 ppm in fat, and 0.022 ppm in skin with fat.

The levels of extractable residues in eggs and tissues of hens dosed at 33.4 ppm were mostly ≤0.01 ppm. Prohexadione was the major residue identified in egg yolks (12.5% TRR, 0.003 ppm), egg whites (2?.3% TRR, 0.004 ppm), liver (14.9% TRR, 0.005 ppm); and kidney (?3.2% TRR, 0.062 ppm) (Table 4). Tricarballylic acid was identified in kidney (15.5% TRR, 0.073 ppm). An unknown component, designated as Metabolite 1, was resolved in egg whites (71.5% TRR, 0.010 ppm), egg yolks (2.3% TRR, 0.001 ppm), and liver (18.2% TRR, 0.006 ppm). Attempts to identify Metabolite 1 using a combination of techniques including acid, base, and/or enzyme hydrolysis, methylation, HPLC, GC/MS and LC/MS were unsuccessful; further attempts were not performed because of insufficient material.

Table 4. Summary of radioactive residues characterized/identified in milk and tissues of laying hens orally dosed with [^{14}C]prohexadione-calcium at 33.4 ppm for 5 consecutive days.

Fraction	Egg yolk, Day 5 (TRR = 0.022 ppm)		Egg white, Day 5 (TRR = 0.014 ppm)		Kidney (TRR = 0.471 ppm)		Liver (TRR = 0.030 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Prohexadione	12.5	0.003	27.3	0.004	13.2	0.062	14.9	0.005
Tricarballylic acid	--	--	--	--	15.5	0.073	--	--
Total identified	**12.5**	**0.003**	**27.3**	**0.004**	**28.7**	**0.135**	**14.9**	**0.005**
Characterized								
Metabolite 1	2.3	0.001	71.5	0.010	--	--	18.2	0.006
Unknown peaks	13.8	<0.005	5.1	<0.002	5.4	<0.025	1.7	<0.001
Aqueous	12.7	0.003	5.6	0.001	6.6	0.031	17.6	0.005
Petroleum ether extract	12.1	0.003	0.8	<0.001	0.3	0.001	2.3	0.001
Semi-solid gel; macromolecules	--	--	--	--	9.3	0.044	--	--
Pronase hydrolysate	--	--	--	--	8.4	0.040	--	--
Total identified/characterized	**53.4**	**0.015**	**110.3**	**0.018**	**58.7**	**0.276**	**54.7**	**0.018**
Nonextractable	42.9	0.009	11.0	0.002	22.0	0.104	36.8	0.011

[a] See Figure 1 for chemical names and structures of identified metabolites.

Livestock metabolism conclusions:
The qualitative nature of the residue in livestock is adequately understood. In ruminants, prohexadione is metabolized to despropionyl prohexadione metabolites or precursors and then to despropionyl prohexadione. The subsequent metabolism of despropionyl prohexadione then yields low molecular weight carboxylic acid and finally incorporation into naturally occurring products such as sugars, lipids and proteins. In poultry, prohexadione is metabolized to tricarballylic acid and then to natural products such as proteins.

Magnitude of the Residue.

Peanuts:
Residues of prohexadione-calcium were <0.05-0.896 ppm in/on peanut nutmeat and <0.05-0.539 ppm in/on peanut hay harvested 25 days (the proposed PHI) following the last of three broadcast foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season; 1x). The results of the peanut field trials support the proposed tolerances of 1.0 ppm in/on peanut nutmeat and 0.6 ppm in/on peanut hay.

Pome fruits:
Residues of prohexadione-calcium were <0.05-2.631 ppm in/on apples harvested 45-46 days (the proposed PHI) following the last of two broadcast foliar applications of the 27.5% DF formulation at 0.85 lb ai/A/application (1.7 lb ai/A/season; 1x). Residues of prohexadione-calcium were 0.23-0.99 ppm in/on pears harvested 44-45 days following a single broadcast foliar application of the 27.5% DF formulation at 1.7 lb ai/A (1x). The petitioner has provided adequate residue data reflecting the maximum proposed use pattern for prohexadione-calcium on pome fruits. **Pending submission of confirmatory storage stability data,** the residue data submitted for apples and pears support the establishment of the proposed crop group tolerance for residues of prohexadione-calcium in/on pome fruits at 3.0 ppm.

Residues of the oxidative metabolite BX 112-15, which was detected in the apple metabolism study, were less than the LOQ (<0.05 ppm) in all treated samples of apple and pear harvested 45 days following treatment at 1x the maximum proposed seasonal application rate. The petitioner proposes that these data represent field conditions, and therefore, the metabolite BX 112-15 is not a residue of concern.

Ruminants:

Dairy cows were orally dosed once daily with prohexadione-calcium at 8, 24, or 80 ppm (4x, 12x, and 40x, respectively, the anticipated maximum dietary burden of 2.0 ppm for dairy cattle and ~2x, ~7x, and ~24x, respectively, the anticipated maximum dietary burden for beef cattle) for 29 consecutive days. Animals were sacrificed within 4.5 hours of the final dose, except for two animals from the highest dose group which were maintained on a "no-treatment diet" after the 29th day of dosing and were sacrificed after 2 and 5 days of withdrawal. The results of the study suggest that residues of prohexadione-calcium are not likely to transfer to milk (including cream and skim milk), fat, liver, and muscle when the chemical is used according to the proposed use directions (Table 5). However, residues of prohexadione-calcium are expected to transfer to liver and kidney. By extrapolation of the average residues at ~2x, ~7x, and ~24x the anticipated maximum dietary burden for beef cattle, HED concludes that the proposed tolerance levels are 0.1 ppm for residues of prohexadione-calcium in cattle kidney and 0.05 ppm for meat byproducts (except kidney).

Cow's kidney and liver samples were additionally analyzed for residues of despropionyl prohexadione, a metabolite identified in the kidney and liver of goats from the ruminant metabolism study. At the lowest feeding level, residues of despropionyl prohexadione in cow's kidney and liver were each nondetectable (<0.05 ppm). These data suggest that residues of the despropionyl metabolite are not likely to transfer to cow's kidney and liver when the chemical is used according to the proposed use directions.

Table 5. Residues of prohexadione-calcium and despropionyl prohexadione in dairy cattle matrices following oral administration of prohexadione-calcium at target feeding levels of 8 ppm, 24 ppm, and 80 ppm for 29 consecutive days.

Target Dose Level (ppm)	Dosing or Sampling Day	Uncorrected Residues (ppm) [a]	
		Prohexadione-calcium	Despropionyl prohexadione (BW 125-5376)
Milk			
80	1	<0.01 (5)	Not analyzed (N/A)
	10	<0.01 (4), 0.013 [b]	N/A
	32	<0.01	N/A
	33	<0.01	N/A
Cream			
80	28	<0.01 (4)	N/A

(continued; footnotes follow)

Target Dose Level (ppm)	Dosing or Sampling Day	Uncorrected Residues (ppm) [a]	
		Prohexadione-calcium	Despropionyl prohexadione (BW 125-5376)
Skim Milk			
80	28	<0.01 (4)	N/A
Fat			
8	29	<0.05 (3)	N/A
24	29	<0.05 (3)	N/A
80	29	<0.05, 0.056, 0.079	N/A
	31	<0.05	N/A
	34	<0.05	N/A
Kidney			
8	29	0.09, 0.097, 0.312	<0.05, <0.05, <0.05
24	29	0.431, 0.638, 0.837	0.080, 0.087, 0.094
80	29	1.66, 2.74 [b], 4.65 [b]	1.78, 4.96 [b], 5.14 [b]
	31	<0.05	<0.05
	34	<0.05	<0.05
Liver			
8	29	<0.05 (3)	<0.05 (3)
24	29	<0.05, 0.05, 0.051	<0.05, <0.05, <0.05
80	29	<0.05, 0.077, 0.208	<0.05, <0.05, <0.05
	31	<0.05	<0.05
	34	<0.05	<0.05
Muscle			
8	29	<0.05 (3)	N/A
24	29	<0.05 (3)	N/A
80	29	<0.05 (2), 0.081	N/A
	31	<0.05	N/A
	34	<0.05	N/A

[a] Each residue value represents a single sample (individual cow) unless otherwise noted in parentheses; residue values for prohexadione-calcium and despropionyl prohexadione are listed respectively.

[b] The highest value of replicate injections is reported.

Poultry:

A poultry feeding study was not submitted with the subject petition. Based on the results of the poulty metabolism study and the maximum theoretical dietary burden, HED has concluded that there is no reasonable expectation of finite residues [§180.6(a)(3)] in eggs and poultry tissues.

Codex Issues

There is neither a Codex proposal, nor Canadian or Mexican limits for residues of prohexadione-calcium in/on plant or animal commodities. Therefore, no compatibility issues exist with regard to the proposed U.S. tolerances discussed in this petition.

2. TOXICOLOGY DATA

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL = N/A UF = N/A	Not required; No appropriate single dose endpoint.	N/A
	Risk Assessment is NOT required.		
Chronic Dietary	NOAEL = 80 UF = 100	Moderate cortical areas of dilated basophilic tubules in the kidneys and decreased potassium levels seen at the LOAEL of 400 mg/kg/day	Subchronic and Chronic Toxicity Studies - Dog
		Chronic RfD = 0.80 mg/kg/day	
Dermal, Short-Term[a]	Oral Maternal NOAEL = 100 MOE = 100	Premature deliveries seen at maternal LOAEL of 350 mg/kg/day	Developmental Toxicity - Rabbit
Dermal, Intermediate-Term[a]	Oral NOAEL = 80 MOE = 100 (Occupational)	Moderate cortical areas of dilated basophilic tubules in the kidneys and decreased potassium levels seen at the LOAEL of 400 mg/kg/day	Subchronic Toxicity Study - Dog
Dermal, Long-Term[a]	Oral NOAEL = 80 MOE = 100 (Occupational)	Moderate cortical areas of dilated basophilic tubules in the kidneys and decreased potassium levels seen at the LOAEL of 400 mg/kg/day	Subchronic and Chronic Toxicity Studies - Dog
Inhalation, Short-Term[b]	Oral Maternal NOAEL = 100 MOE = 100 (Occupational)	Premature deliveries seen at maternal LOAEL of 350 mg/kg/day	Developmental Toxicity - Rabbit
Inhalation, Intermediate-Term[b]	Oral NOAEL = 80 MOE = 100 (Occupational)	Moderate cortical areas of dilated basophilic tubules in the kidneys and decreased potassium levels seen at the LOAEL of 400 mg/kg/day	Subchronic Toxicity Study - Dog
Inhalation, Long-Term[b]	Oral NOAEL = 80 MOE = 100 (Occupational)	Moderate cortical areas of dilated basophilic tubules in the kidneys and decreased potassium levels seen at the LOAEL of 400 mg/kg/day	Subchronic and Chronic Toxicity Studies - Dog

[a]A dermal absorption factor of 100% or ?? should be used for risk assessment

[b]An inhalation absorption factor of 100% and a dermal absorption factor of 100%or ?? should be used for route-to-route extrapolation for occupational risk assessments.

Metabolism: In a rat metabolism study (MRIDs 44457770-44457773), [^{14}C-C3 or C5-cyclohexene]BX-112 (>97.6 % a.i.) was administered to Fischer 344 rats (5/sex/dose) as a single oral (gavage) dose at 50 or 500 mg/kg or as a single oral dose at 50 mg/kg following a 14-day pretreatment with BX-112 at 50 mg/kg. In addition, four male and female bile-cannulated Fischer 344 rats were administered a single oral dose of [^{14}C]BX-112 at 50 mg/kg.

Analyses of urine and fecal extracts identified and/or characterized 55.8.4-75.3% of the dosed radioactivity for each dose group. Metabolism of BX-112 was qualitatively and quantitatively similar between sexes, although there were minor quantitative differences between males and females in the high-dose and repeated low-dose groups. Metabolism was also qualitatively similar between dose groups.

In the low-dose groups (with or without pretreatment), the major metabolite in excreta was the free acid metabolite, KI-2817 (38.3-53.7% dose), which was excreted primarily in the urine (20.2-31.6% dose). The only other significant component isolated from excreta of low-dose rats was the putative base-labile conjugate of KI-2817, which accounted for 15.5-21.3% of the dose in urine. In the high-dose group, KI-2817 (60.9-68.0% dose) was also the major metabolite in excreta. However, the majority of KI-2817 (53.4-64.6% dose) from high-dose rats was recovered in the feces rather than in urine, and levels of both KI-2817 and its putative base-labile conjugate were lower (3.4-7.5% dose) in urine. Minor amounts of KI-5376 (1.1-2.3% dose) were also identified in the feces of high-dose rats. KI-2817 was also identified as the principal metabolite (28.5-76.6% of tissue radioactivity) in liver and kidney extracts from low and high-dose males.

3. ENVIRONMENTAL FATE DATA (From Memo, Iwona L. Maher; D260214)

Prohexadione calcium is not expected to persist long in the environment based on laboratory studies submitted. The major route of prohexadione calcium dissipation is oxidative mineralization to CO_2 in the soil. The nonlinear first order kinetics $T_{1/2}$ of aerobic degradation in the sandy loam soil was 1.4 days (value used for modeling, r^2 = 0.98) while the linear $T_{1/2}$ was 9.8 days (r^2 = 0.73, MRID 44457785). Prohexadione calcium hydrolysis is pH dependent; it hydrolyzes fairly rapidly at pH 5 and does not hydrolyze at pH

9 ($T_{1/2}$ = 4.4 days; $T_{1/2}$ = 65 days; in pH 9 did not hydrolyze . It photodegrades with a slower rate in all aquatic environments ($T_{1/2}$ = 23.2 days, $T_{1/2}$ = 9.9 days; hydrolysis corrected half-lives). In anaerobic soil/aquatic conditions prohexadione calcium is stable ($T_{1/2}$ = 117 days) and it is also photolytically stable on soil ($T_{1/2}$ = 32.4 days in an irradiated sample and 3.1 days in a dark control, MRID 44457784). The registrant-proposed degradation pathway indicates that prohexadione calcium degrades to despropionyl prohexadione which degrades further into tricarballylic acid and citric acid, two naturally occurring substances. The two acids are subsequently mineralized to CO_2.

According to the McCall classification (McCall *et al.*, 1980) prohexadione calcium showed very high mobility potential in the sand soil (the linearized sorption coefficient (K_{d1}) = (K_1)*(Adj. Factor) 0.50; K_{oc} = (K_{d1}*100) / %OC = (K_{d1}*100*1.724) / %OM = 173; adjustment factors derived based on van Genuchten et al., 1977, and Parker and Jardine,1980), low mobility potential in the loamy sand soil (K_1 = 9.1; K_{oc} = 1428), and moderate mobility in all other soils (the clay soil's K_{d1} = 2.8 and K_{oc} = 155; the loam soil's K_{d1} = 11.0 and K_{oc} = 421, MRID 44457787). There was no linear relationship between the soil organic carbon and the K_d values for different soils (r^2 = 0.28; MRID 44457787). Detection of prohexadione calcium concentrations (four single replicates at 0.015 - 0.367 ppm, LOQ = 0.01 ppm) in 6- to 12-inch soil depths in the field dissipation study (apple growing areas of NY, CA, and OR, MRID 44721213) is indicative of leaching as a possible route of prohexadione calcium dissipation. There was no residue detection below 12 inches at concentrations greater than or equal to 0.01 ppm.

Although prohexadione calcium is expected to be mobile in some soils, it should not pose a threat to ground water because of its rather rapid degradation. Some environmental conditions, however, such as excess precipitation occurring immediately after application or during preferential flow conditions in structural soil, could allow for its rapid runoff or leaching.

4. QUESTIONS TO THE MARC

1. Is there any scientific objection to establishing the tolerance and conducting risk assessment in terms of prohexadione-calcium *per se*?

2. Are additional prohexadione-calcium metabolites in **RACs** at the

levels reported of special toxicological concern? If so, which one(s). Do they warrant inclusion in the tolerance regulation? Separate regulation? Inclusion in the dietary risk assessment? Additional metabolism studies? Toxicological studies?

3. Are additional prohexadione-calcium metabolites in **drinking water** at the levels reported of special toxicological concern? If so, which one(s)? Do they warrant inclusion in the dietary risk assessment?

ATTACHMENT I- Figure 1: Chemical names and structures of prohexadione and its metabolites identified in plant and animal metabolism studies.

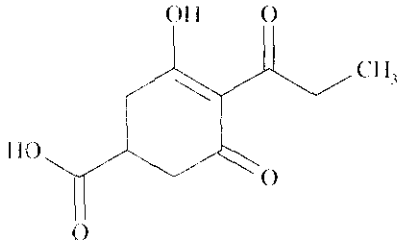
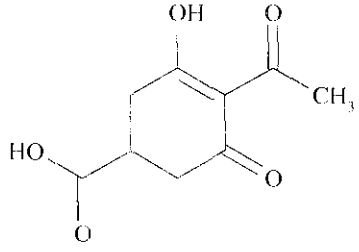
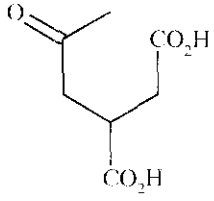
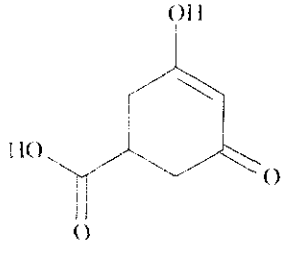
ATTACHMENT II- Figure 2: The metabolic pathways of prohexadione-calcium in apples.

ATTACHMENT III- Figure 3: The metabolic pathways of prohexadione-calcium in peanuts.

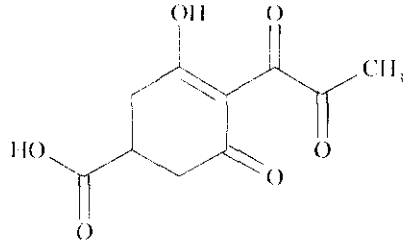
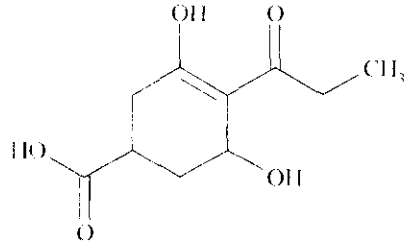
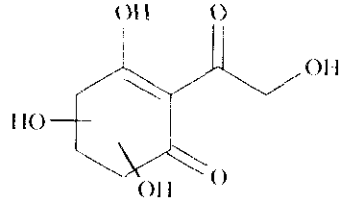
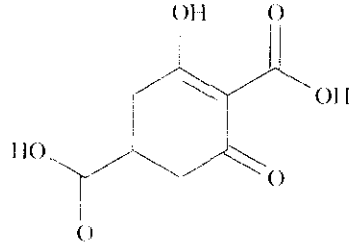
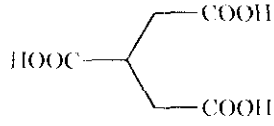
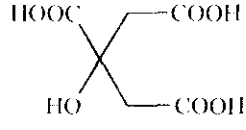
ATTACHMENT IV- Figure 3: The metabolic pathways of prohexadione-calcium in rats.

cc: 8F04941, Kramer, HED Metabolism Committee File (G. Kramer), Cynthia Giles-Parker(RD),
RDI: M. Morse (//00), Team (2/3/00)
C.F. Kramer: 14V:CM#2:(703)305-5079:"5C0C

Figure 1. Chemical names and structures of prohexadione and its metabolites identified in plant and animal metabolism studies.

Common Name or Company Code Chemical Name	Structure	Substrate
Prohexadione (BAS 125 W, free acid; BAS 9054 W 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate		Apples Peanut nutmeats, hulls, and hay Goat milk, kidney, liver, muscle, and fat Hen egg yolk and whites, kidney, and liver
BX 112-I5 (BW125-31F		Apples
BX 112-M10		Apples
Despropionyl prohexadione (referred to as **BW 125-5376** in the ruminant feeding study)		Apples Peanut hulls and hay Goat milk, kidney, liver, muscle, and fat

(continued)

Common Name or Company Code Chemical Name	Structure	Substrate
Dioxopropyl prohexadione		Peanut nutmeats, hulls, and hay
27F2-B (and 45F2-A) 3,5-Dimethoxy-4-propionyl-methyl benzoate		Apples
25F1-A 2-Hydroxyacetyl-3-methoxy-dihydroxy-1-cyclohexenone		Apples
27F2-A (and 27F1-A) 3,5-Dioxocyclohexane-1,4-dimethyl-dicarboxylate		Apples
Tricarballylic acid (TCA)		Apples Peanut nutmeats, hulls, and hay Hen kidney and liver.
Citric acid		Apples

* BAS 125W was used interchangeably by the petitioner to refer to prohexadione-calcium or prohexadione (free acid).

Figure 2. Metabolism Pathway for Prohexadione-Calcium in Apples

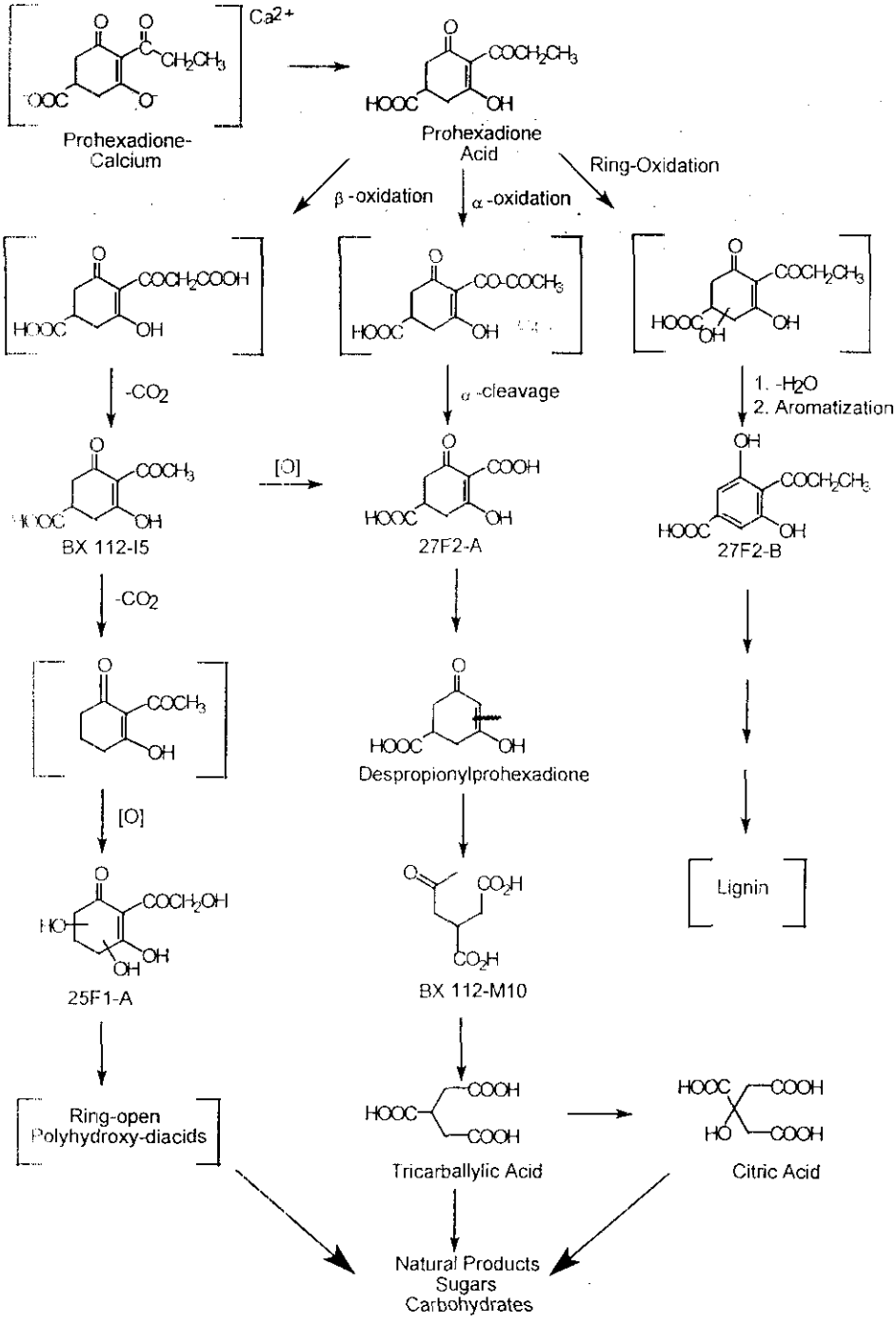


Figure 3. Metabolism Pathway for Prohexadione-Calcium in Peanuts and Animals

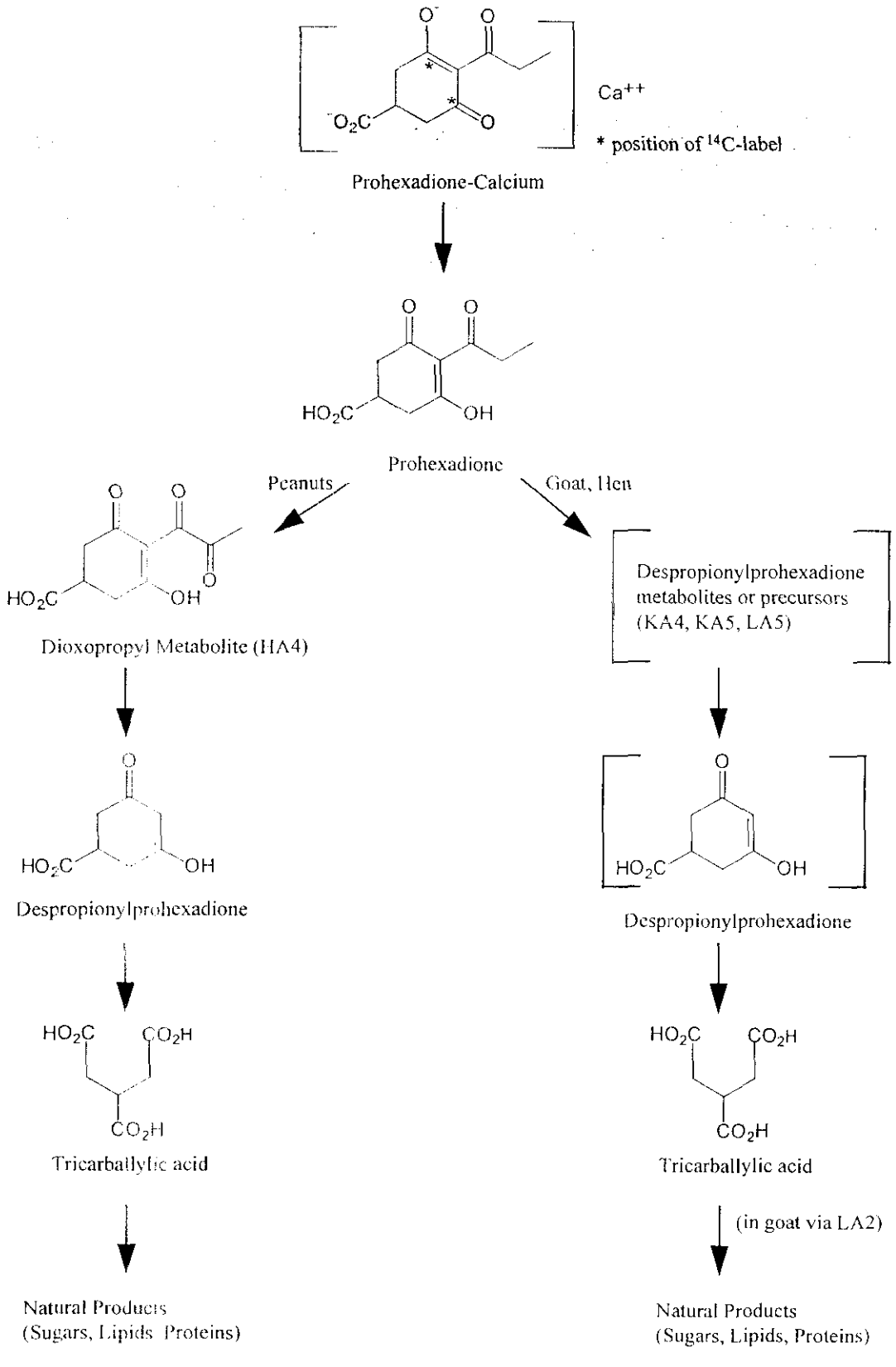
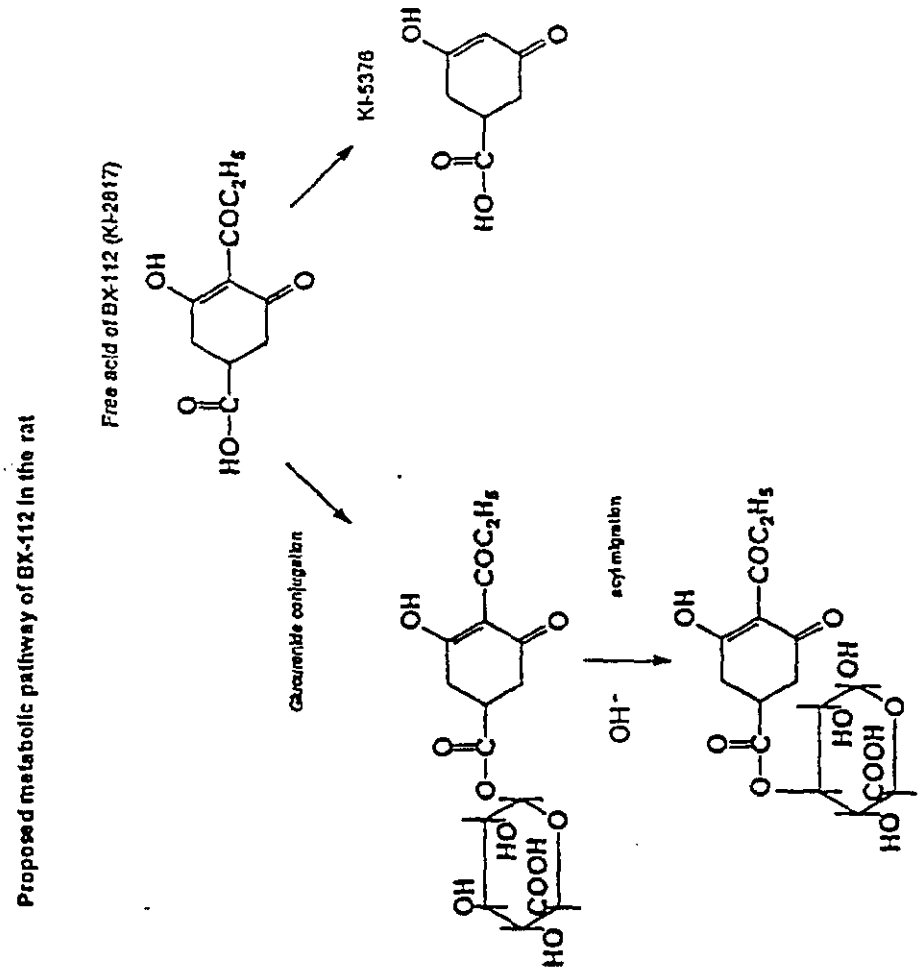


Figure 4


Proposed metabolic pathway of BX-112 in the rat
Free acid of BX-112 (KI-2817)
KI-5378
Glucuronide conjugation
acyl migration
OH-

13544

R127652

Chemical:	**Cyclohexanecarboxylic acid, 3,5-dioxo-4-(1-oxopropyl)-, ion(1-), calcium, calcium salt**
	PC Code: 112600
HED File Code:	**21400 MARC Reports**
Memo Date:	**2/14/2000**
File ID:	**DPD262930**
Accession #:	**412-06-0199**

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